

Fatty Acids and Lipids Biosynthesis in Yeast and Plants

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Fatty acids and lipids biosynthesis in yeast and higher plants have been detail discussed in this article. Synthesis of lipids implicates a composite sequence of biochemical reactions. Understanding different lipid classes' synthesis improves the ability to experimentally modify lipid desaturation pathways in order to produce oils with enhanced features for food and manufacturing usages. However, ω -3 fatty acids biosynthesis and their accumulation into different lipid classes are not entirely understood. Fatty acids are synthesized inside the plant chloroplast via fatty acid synthase complex up to a chain length of 16C. Successive elongation and other alterations of fatty acyl residues and TAG association are occurred in both cytoplasm and the endoplasmic reticulum (ER). Biosynthesis of lipids in higher plants as well as in yeast involves two pathways that may or may not coexist depending on the species. Prokaryotic pathway leads to the synthesis of lipids by using only plastidial enzymes, while the eukaryotic pathway involves adjacent collaboration among the ER and the chloroplasts. The comprehensive roles of desaturases and acyltransferases in lipid synthesis have been deliberated.

Key words: Fatty Acids, Lipids, Yeast, Plants, Prokaryotic, Eukaryotic.

The essential classes of yeast storage lipids are Triacylglycerols (TAG) and steryl esters (SE). SE is formed by binding of only one acyl chain to the hydroxyl group at Carbon 3 position of the sterol, TAG contain three fatty acids esterified to the glycerol backbone¹. Seed oils are composed primarily of triacylglycerols (TAG's), which are glycerol esters of fatty acids. Commercial vegetable oils are produced primarily from six major oil crops (soybean, oil palm, rapeseed, sunflower, cotton seed, and peanut). Vegetable oils are used predominantly (90%) for human consumption as margarine, shortening, salad oils, and frying oil².

The remaining 10% is used for non-food applications such as lubricants, oleo-chemicals, biofuels, detergents, and other industrial applications. The desired characteristics of the oil used in each of these applications vary widely, particularly in terms of the chain length and number of double bonds present in the fatty acids making up the TAG's. Plants manipulate these properties in order to control membrane fluidity and temperature sensitivity. The same properties can be controlled through agricultural biotechnology to produce oils with improved characteristics for food and industrial uses. The primary fatty acids in the TAG's of oilseed crops are 16 to 18 carbons in length and contain 0 to 3 double bonds. Palmitic acid (16:0 [16 carbons: 0 double bonds]), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) predominate. The number of double bonds, or degree of saturation, determines the melting temperature, reactivity, cooking performance, and health attributes of the resulting oil. For example, oils that are relatively high in polyunsaturated fatty

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acids (18:2 and 18:3) such as soybean oil oxidize readily, resulting in poor taste, and will not solidify at room temperature, making them unsuitable for margarine and shortening applications. Such oils are therefore chemically hydrogenated to reduce unsaturation, a process that adds cost and may have undesirable health consequences³. On the other hand, an even higher percentage of polyunsaturated fatty acids would be advantageous for many industrial applications such as varnishes, coatings, and drying oils⁴. Thus, the ability to regulate fatty acid desaturation to fit particular oil applications is desirable. Humans required increasing their nutritional ω -3 fatty acid levels. Oils high in ω -3 fatty acids are beneficial as drying oils in several products such as printing inks. So markets need soybean oil to be engineered to produce such nutritionally and industrially valued compounds at relatively minor production costs. Alteration of plant lipid biosynthesis is not restricted to using genes from the plants themselves but interspecies transfer is possible from completely unrelated plant species^{5,6,7,8,9}. The ability to experimentally modify plant lipid desaturation pathways has been proven in several instances. For example, traditional crop breeding has resulted in dramatic increases in stearic acid in safflower and soybean¹¹. Using genetic engineering, researchers at Calgene, Inc. were successful in modifying the seed oil profile of canola, *Brassica napus*¹². This was accomplished by introducing an antisense copy of the gene encoding stearoyl-ACP (Δ 9) desaturase under the control of a seed-specific promoter, resulting in decreased levels of this enzyme in the seed. However expression of Acyl-ACP desaturase from *Pelargonium xhortorum* under control of a seed specific promoter in *Arabidopsis* resulted in the accumulation of an unusual monoene in only 1-15% of seed fatty acids. These levels of fatty acid production were not correlated with the level of desaturase expression^{10,13,14}. In order to reach these ultimate goal researchers should identify and evaluate the synthesis of ω -3 fatty acids in oil seeds, and its accumulation in TAG. Flax (*Linum usitatissimum*) oil content ranges from 40-52%, 40-64% of this oil is linolenic acid (according to flax variety)^{15,16,17}. The oil content of *Dracocephalum moldavica* is 25-30% and linolenic acid forms 59-68% of it^{18,19,20,21,22}. The oil content of *Perilla*

frutescens is 35-45%, and contains 50-64% 18:3^{10,14,18,23}. Accumulation of fatty acids including ω -3 fatty acids in plant oils is controlled at least in part by acyltransferases especially diacylglycerol acyltransferase (DGAT)^{13,24,25}. These and other studies testify to the plasticity of plant seed oil desaturation pathways and suggest that other major changes in oil composition will be possible and will be tolerated by the plant.

Fatty acid biosynthesis

The synthesis of lipids involves a complex series of biochemical reactions (Figure 1)^{4,5,22,26,27}. Fatty acids are manufactured within the plant chloroplast by the fatty acid synthase complex up to a chain length of 16C⁴. Subsequent elongation and other modifications of fatty acyl residues and triacylglycerol (TAG) assembly are localized in the cytoplasm and the (ER). The first committed step in fatty acid synthesis is the formation of malonyl-CoA by acetyl-CoA carboxylase (ACCase), which is considered to be a rate-limiting step in fatty acid biosynthesis²⁸. Synthesis of the 18C fatty acid from acetyl-CoA and malonyl-CoA precursors is catalyzed by seven enzymes^{2,29,30}, which constitute the fatty acid synthase (FAS) complex. The central carbon donor for fatty acid synthesis is the malonyl-CoA produced by ACCase from CO₂ and acyl-CoA. However, before entering the fatty acid synthesis pathway, the malonyl group is transferred from CoA to a protein cofactor, acyl carrier protein (ACP). Three condensing enzymes then utilize malonyl-ACP as the 2C donor for elongation of the growing acyl chain. The first condensation of acetyl-CoA and malonyl-ACP to form a 4C product is catalyzed by 3-ketoacyl-ACP synthase III^{31,32}. The second condensing enzyme is KASI which is responsible for the production of 6C to 16C fatty acids. Subsequently palmitic acid (16:0) is removed from its acyl carrier protein (ACP) via the action of the thioesterase enzyme, transferred to the cytoplasm, and converted into palmitoyl-CoA. In addition, the 16:0-ACP could be elongated in the chloroplast to form stearic acid (18:0) by the KASII enzyme. The fatty acid biosynthesis pathway produces saturated fatty acids, but in most plant tissues over 75% of the fatty acids are unsaturated²⁶. The first desaturation step in the chloroplast forming oleic acid is catalyzed by the Δ 9-stearoyl-ACP desaturase enzyme^{33,34}. This fatty acid desaturase

is unique to the plant kingdom since it is soluble and all other known eukaryotic desaturases are integral membrane proteins^{26,35}. Then thioesterase released oleic acid from ACP and transported into the cytoplasm. However, how the free fatty acids are transported out of the plastid is unknown, but this may happen through a simple diffusion across the envelope membrane²⁶. On the outer membrane of the chloroplast envelope, the acyl-CoA synthetase is thought to assemble the acyl-CoA thioester that is then available for acyltransferase reactions for glycerolipid synthesis in the ER. How the acyl-CoA moves from the outer chloroplast envelope to the ER is also unknown, but it may involve acyl-CoA binding proteins²⁶.

Triacylglycerol the major glycerolipid

The biosynthesis of lipids in higher plants as well as in yeast involves two pathways that may or may not coexist depending on the species (Figure 2). The prokaryotic pathway leads to the synthesis of lipids by using only plastidial enzymes, while the eukaryotic pathway requires close cooperation between the endoplasmic reticulum (ER) and the chloroplasts². Palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1) are synthesized before being incorporated to glycerolipids while linoleic (18:2) and linolenic (18:3) acids are formed on glycerolipids by the action of membrane-bound desaturases of the chloroplasts and ER²⁶. In higher plants, there are two major pathways for the synthesis of glycerolipids: the “prokaryotic pathway” of the chloroplast inner envelope, and the “eukaryotic pathway”, which begins with phosphatidic acid (PA) synthesis in the ER. Fatty acids synthesized *de novo* in plastids either may be used directly for production of chloroplast lipids by the prokaryotic pathway or may be exported to the cytoplasm as CoA esters, which are then incorporated into lipids in the ER by an independent set of acyltransferases. Both pathways are initiated by the synthesis of 16:0-ACP by the fatty acid synthase in the plastid. This 16:0-ACP may be elongated to 18:0-ACP by KASII and then desaturated to 18:1-ACP by a soluble desaturase^{34,37,38,39}, so that 16:0-ACP and 18:1-ACP are the primary products of plastid fatty acid synthesis. These thioesters may be used within the plastid for the synthesis of PA, or they may be hydrolyzed to free fatty acids, which move through

the plastid envelope to be converted to CoA thioesters in the outer envelope membrane by acyl-CoA synthetase^{2,26,30,40}. Because of the substrate specificities of the plastid acyltransferases^{41,42}, the PA is made by the prokaryotic pathway has 16:0 at the *sn*-2 position and, in most cases, 18:1 at the *sn*-1 position; the first enzyme of the prokaryotic pathway, acyl-ACP:glycerol-3-phosphate acyltransferase, is highly specific⁴², while the second acylation, catalyzed by a membrane-bound acyl-ACP: 1-acyl-glycerol-3-phosphate acyltransferase (LPAT)^{40,42}. The PA is used for the synthesis of PG or is converted to DAG by the PA-phosphatase located in the inner plastid envelope. DAG pool can be used as a precursor for the synthesis of the other major plastid membrane glycerolipids such as MGD, DGD, and SL⁴³.

However, the acyl-CoA-specific acyltransferases of the ER produce PA that is highly enriched in unsaturated 18C fatty acids at the *sn*-2 position and if 16:0 is present it will be transferred to the *sn*-1 position^{42,44}. The microsomal LPAT almost completely excludes palmitoyl-CoA and stearoyl-CoA from the *sn*-2 position of the PA^{42,44}. However, when 16:1-CoA is produced, it is mainly incorporated into the *sn*-2 position⁴⁵. The PA is used as a precursor for the phospholipids such as PC, PE, and PI, which are characteristic of the various extraplastidial membranes.

In addition, the DAG moiety of the PC could be returned to the chloroplast envelope where it enters the DAG pool and used in the synthesis of the plastidial lipids^{46,47}. Evidence from several *Arabidopsis* mutants indicates that lipid exchange between the ER and the chloroplast is reversible to some extent^{48,49} because extraplastidial membranes in mutants deficient in ER desaturases contain polyunsaturated fatty acids derived from the chloroplasts. The relative flux through the two pathways varies from species to species^{46,47,50}. In all higher plants, chloroplast PG is synthesized only from the prokaryotic PA⁴⁶, while synthesis of the other chloroplast lipids has large contributions from the eukaryotic pathway⁵¹. DAG-phosphate is converted to DAG by phosphatidic acid phosphatase (PAP). DAG has two fates. It can be used to form TAG or it can be used in a freely reversible PC-DAG interconversion to enter membrane lipid synthesis^{41,42}. Finally, DAG acyltransferase (DGAT), which is the enzymatic

step for TAG synthesis⁵⁰, adds the third acyl chain into DAG. The TAG thus formed is deposited in lipid bodies. However steryl esters is synthesized in yeast through esterification of sterols with a long chain fatty acid catalyzed by acyl-CoA:sterol acyltransferase (ASAT) enzyme which is encoded by the two different genes (*ARE1* and *ARE2*) in *Saccharomyces cerevisiae*, also correlated genes encoding ASAT activity enzymes occur in different higher eukaryotes^{52,53,54,55}.

Desaturation and desaturases

Biosynthesis of ω -3 fatty acids and their accumulation in plant oils is not fully understood. It is known that ω -3 fatty acids are synthesized from ω -6 fatty acid precursor membrane lipids in plastids and the ER by ω -3 desaturases. More than 20 ω -3 desaturase gene sequences have been reported mostly from higher plants including several from soybeans but the metabolic contributions to oil ω -3 fatty acid content are not yet known. The transcripts of both Δ -12 and ω -3

fatty acid desaturases, which are involved in the desaturation of oleic and linoleic acids respectively, accumulate in the *Arabidopsis rfc4* mutant (regulator of fatty acid composition mutant). In the *rfc4* mutant, over accumulation of 18:3 found to be correlated with increase in ω -3 desaturase mRNA over the wild type levels which suggest that polyunsaturated fatty acid accumulation is controlled by structural genes for fatty acid desaturases^{56,57}.

Accumulation of 18:3 in triacylglycerols (TGs) can be controlled at least in part by diacylglycerol acyltransferases (DGATs) also bound to the ER. DGATs can affect ω 3 fatty acid accumulations in TG by showing higher affinities (lower Kms) for both 18:3-CoAs and diacylglycerols containing 18:3. DGATs can also influence level of triacylglycerol accumulation²⁴. The pathways involved in the formation of TG in seeds are not fully understood, and the glycerol-3-phosphate pathway (Kennedy pathway)³⁶ has been proposed

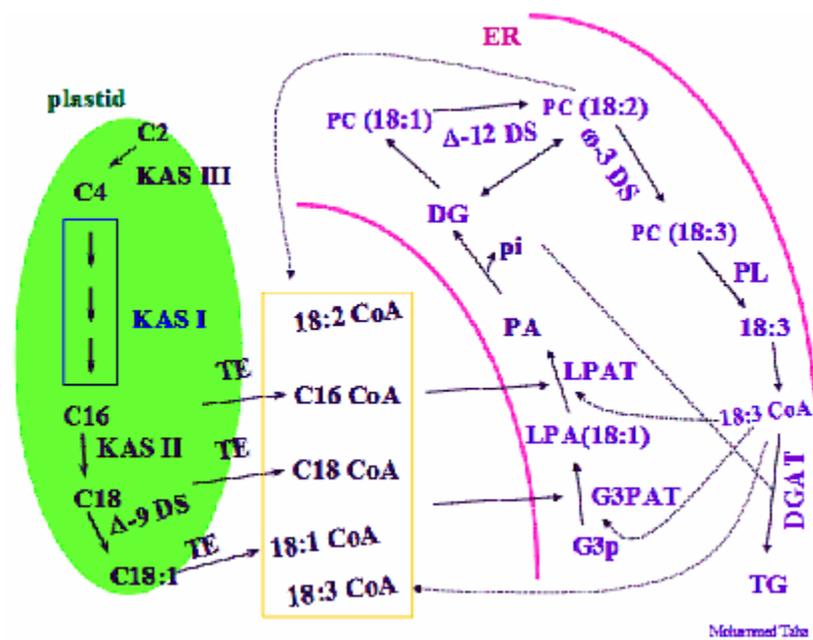


Fig. 1. *De novo* fatty acid biosynthesis from C2 (acetyl-CoA) originally found in the plastid, and then the synthesized fatty acids are delivered into the cytosol where they are further modified. Triacylglycerol (TAG) assembly is catalyzed by the enzymes of the Kennedy pathway³⁶. Where, CoA, coenzyme A; DGAT, diacylglycerol acyltransferase; Δ -9 DS, Δ -9-stearoyl-ACP desaturase; Δ -12 DS, Δ -12-oleate desaturase; G3P, glycerol-3-phosphate; G3PAT, G3P acyltransferase; LPAT, lysophosphatidic acid acyltransferase; ω -3 DS, omega-3 linoleate desaturase; PA, phosphatidic acid; PC, phosphatidylcholine; DG, diacylglycerol; TG, triacylglycerol; TE, acyl-ACP thioesterase; KASIII, 3-ketoacyl-ACP synthase III; KASI, 3-ketoacyl-ACP synthase I; KASII, 3-ketoacyl-ACP synthase II; and PL, phospholipase

for TG biosynthesis containing common saturated and unsaturated FA in soybean⁵⁸. The first desaturation step then takes place in the chloroplast to form oleic acid (18:1 Δ^9 [where Δ^9 indicates that the position of the double bond is between carbons 9 and 10]); this is catalyzed by the Δ^9 -stearoyl-ACP desaturase using 18:0-ACP as substrate³³. The oleic acid is then released from its ACP by a thioesterase converted to a CoA thioester and transported into the cytoplasm. In all plant tissues, the major glycerolipids are first synthesized using

only 16:0 and 18:1 acyl groups. Subsequent desaturation of the lipids to the highly unsaturated forms typical of the membranes of plant cells is carried out by membrane-bound desaturases of the chloroplast and the ER^{2,59}. In ER the subsequent desaturation steps to linoleic acid (18:2 $\Delta^9, 12$) and linolenic acid (18:3 $\Delta^9, 12$, and 15) are catalyzed by Δ^{12} desaturase (also known as 6-oleate desaturase) and ω -3 desaturase (also known as Δ^{15} -linoleate desaturase) (Figure 1). The biosynthesis of 18:3 in developing seed tissues

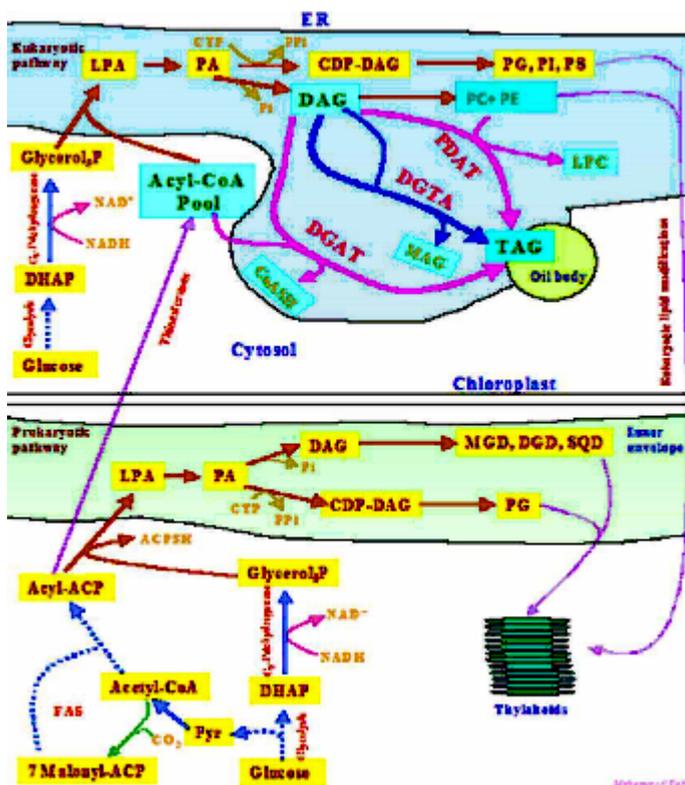


Fig. 2. Prokaryotic and eukaryotic pathways of glycerolipids synthesis. The prokaryotic pathway takes place in plastids and predominantly esterifies palmitate to the *sn*-2 position of lysophosphatidic acid (LPA). The eukaryotic pathway occurs outside the plastid, mainly in the ER and predominantly esterifies 18C fatty acids to the *sn*-2 position of glycerolipids. In both the prokaryotic and eukaryotic pathways acyl-ACP or acyl-CoA is condensed with glycerol 3-phosphate by GPAT, glycerol 3-phosphate acyltransferase, producing LPA, lysophosphatidic acid, which is converted into PA, phosphatidic acid, by LPAT, lysophosphatidic acyltransferase, then PA is subsequently converted into the predominant glycerolipid classes in both plastids and ER. Lipids move from the ER to the other organelles, including the outer envelope of plastids then into the inner envelope by an unknown mechanism for further modifications by the replacement of the head groups and the actions of desaturases. FAS, fatty acid synthase; CoA, coenzyme A; DGAT, diacylglycerol acyltransferase; G3P, glycerol-3-phosphate; G3PAT, G3P acyltransferase; LPA, lysophosphatidic acid; PA, phosphatidic acid; PC, phosphatidylcholine; DG, diacylglycerol; TG, triacylglycerol; PDAT, phosphatidylcholine diacylglycerol acyltransferase; DGTA, diacylglycerol transacylase; CDP-DAG, cytidine diphosphate-diacylglycerol; DGD, digalactosyldiacylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; MDG, monogalactosyldiacylglycerol; SQD, sulfoquinovosyldiacylglycerol; DHAP, dihydroxy-acetonephosphate.

results primarily from consecutive desaturations on the ER membrane. 18:1, the primary product of plastid fatty acid biosynthesis is desaturated to 18:2 by an ER D12 desaturase encoded by *FAD2* and 18:2 to 18:3 by an ER bound ω 3 desaturase encoded by *FAD3*⁶⁰. These ER desaturases desaturate fatty acids esterified to phosphatidylcholine (and possibly other phospholipids)²⁶. Microsomal ω 3 desaturase works on 18:2 PC as a main substrate for, however its plastid isoforms use linoleoyl galactolipids or linoleoyl sulfolipids as a main substrate⁶¹. In plants, ω -3 fatty acid desaturation is found to be in two different cell compartments on two different substrate classes^{50,62}. In plastid, the products of *Fad7* and *Fad8* desaturate both 16:2 and 18:2 attached to glycerolipids. However outside of the plastid and mostly on the endoplasmic reticulum the product of *Fad3* (ω -3 desaturase) desaturates 18:2 attached to phosphatidylcholine. In both cases the desaturation reactions require molecular oxygen and an electron donor, which is ferredoxin in the plastid and cytochrome b_5 and cytochrome b_5 reductase in the ER^{38,62}. Plastidial and extraplastidial *Arabidopsis* desaturases have high amino acid sequence similarity. *Arabidopsis FAD3* and *FAD7* have 66% identity⁶⁰. This suggests a relatively recent evolutionary divergence, structural and functional similarities. In addition desaturases share a general membrane topology and a histidine-rich iron-binding domain^{38,62}. Investigation of these desaturases by traditional biochemical approaches has been limited because solubilizing and purifying them have proven to be very difficult⁶³, due to their membrane requirement. Our understanding of the mechanisms and regulation of the chloroplast and ER desaturases has benefited considerably from the characterization of seven classes of *Arabidopsis* mutants, each one deficient in a specific desaturation step^{26,30,64}. Mutations in two loci *fad2* and *fad3*, primarily affect desaturation of the extraplastidial lipids, whereas mutations in the remaining five loci, *fad4*, *fad5*, *fad6*, *fad7*, and *fad8*, affect the chloroplast lipid desaturation. Two of the chloroplast desaturases are highly substrate specific. The *fad4* gene product encodes a Δ -3 desaturase which catalyzes the insertion of a *trans* double bond into the 16:0 esterified to the *sn*-2 position of PG⁶⁵, whereas the *fad5* gene encoded a protein which catalyzes the synthesis of 16:1 on

MGD and possibly DGD⁶⁶. In contrast, the other three chloroplast desaturases act on acyl chains with no apparent specificity for the length of the fatty acid chain (16 or 18 carbon), its point of attachment to the glycerol backbone (*sn*-1 or *sn*-2), or the nature of the lipid head group. The protein encoded by the *fad6* gene is a 16:1/18:1 desaturase⁵¹, whereas two 16:2/18:2 desaturase isozymes are encoded by *fad7* and *fad8* respectively^{67,68,69}. The ER 18:1 (*fad2*) and 18:2 (*fad3*) desaturases act on fatty acids at both the *sn*-1 and *sn*-2 positions of the lipid molecule^{48,49}. These enzymes have been characterized as PC desaturases, but it is possible that they act on other phospholipids as well. However, isolation of most of the genes encoding membrane-bound desaturases has been accomplished due to recent advances in molecular biology and genetics.

ω -3 desaturase enzyme has two isoforms, microsomal, and plastid isoforms, both isomers probably involved in the final trilinolenylglycerol content⁵⁰. In addition to that, the omega-3 fatty acid desaturases are membrane-bound proteins and there are two different microsomal isoforms in *Oryza sativa*⁷⁰. However in *Spinacia oleracea* there is only one chloroplast membrane-bound ω 3 desaturase⁷¹. The desaturase enzymes in higher plants have an N-terminal domain related to cytochrome b_5 ⁷².

Omega-3 desaturase enzyme is the product of *Fad3* gene⁷³. In *Arabidopsis* the *Fad7* locus encodes a chloroplast omega-3 desaturase^{74,75}. However the role of the *Fad8* locus to provide increased ω -3 desaturase activity in plants that are exposed to low growth temperatures⁶⁸. In addition the structural *fad7* gene contained seven introns interrupted the coding sequence of 1338 base pairs and the *fad7* gene product is homologous with *fad3* gene product indicating that *fad7* encoding ω -3 desaturase and the two genes are ancestral genes⁷⁴. *Oryza sativa fad3* is consists of 8 exons and 7 introns⁷⁰.

Acyltransferases

Several DGATs have recently been reported from higher plants. Diacylglycerol acyltransferase enhances seed oil content and seed weight, a mutation in *Arabidopsis* DGAT is correlated with a change in seed fatty acid composition, reduced DGAT activity, and reduced seed TAG content. In addition DGAT cDNA over-

expression increases TAG synthesis which found to be correlated with DGAT transcript levels^{13,24,25}.

In rapeseed, for example, LPAT is most efficient in transferring unsaturated *cis* fatty acids but does not incorporate erucic acid or fatty acids of medium chain length into the sn-2 position during TAG synthesis^{76,77}.

In the ER, TAGs can be formed by the stepwise acylation of glycerol-3-phosphate (G3P) according to the Kennedy pathway (Figure 1)³⁶. The G3P backbone, which is sequentially acylated, is probably derived from the reduction of the glycolytic intermediate dihydroxyacetone phosphate, catalyzed by a soluble G3P dehydrogenase (GPDH) (Figure 2)^{78,79}. The level of *cis*-unsaturated fatty acid in phosphatidylglycerol (PG) from rice leaves was genetically altered from 19.3% in the wild-type to 29.4 and 32.0% by the transformation of *Arabidopsis* plastidial GPAT demonstrating the practical importance of acyltransferases enzymes such as GPAT in the improvement of plant lipids⁸⁰.

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